

Expression of deletion constructs of bovine β -1,4-galactosyltransferase in *Escherichia coli*: importance of Cys134 for its activity

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Bovine β -1,4-galactosyltransferase (β -1,4-GT; EC 2.4.1.90) belongs to the glycosyltransferase family and as such shares a general topology: an N-terminal cytoplasmic tail, a signal anchor followed by a stem region and a catalytic domain at the C-terminal end of the protein. cDNA constructs of the N-terminal deleted forms of β -1,4-GT were prepared in pGEX-2T vector and expressed in *E.coli* as glutathione-S-transferase (GST) fusion proteins. Recombinant proteins accumulated within inclusion bodies as insoluble aggregates that were solubilized in 5 M guanidine HCl and required an 'oxido-shuffling' reagent for regeneration of the enzyme activity. The recombinant β -1,4-GT, devoid of the GST domain, has 30–85% of the sp. act. of bovine milk β -1,4-GT with apparent K_m s for N-acetylglucosamine and UDP-galactose similar to those of milk enzyme. Deletion analyses show that both β -1,4-GT and lactose synthetase activities remain intact even in the absence of the first 129 residues (pGT-d129). The activities are lost when either deletions extend up to residue 142 (pGT-d142) or Cys134 is mutated to Ser (pGT-d129C134S). These results suggest that the formation of a disulfide bond involving Cys134 holds the protein in a conformation that is required for enzymatic activity.

Key words: amino-deleted constructs/disulfide bridge mutants/inclusion bodies/recombinant bovine β -1,4-galactosyltransferase/renaturation

Introduction

The synthesis of complex oligosaccharides is dependent on a class of enzymes called glycosyltransferases. A specific transferase is required for the formation of each of the different disaccharide linkages that are known to be present in the diverse oligosaccharide structures (Roseman, 1970). These enzymes are highly specific for the donor and the acceptor substrates and have been grouped into families according to the type of sugar they transfer (Beyer *et al.*, 1981). Since only few nucleotide sugars are found to be transferred into a growing carbohydrate chain, amino acid sequence homologies would be expected among these families. Surprisingly no extensive sequence similarities have been identified among the deduced amino acid sequences of cDNAs encoding GlcNAc β -1,4-GT, Gal α -2,6-sialyltransferase (ST) and α -1,3-GT (Paulson and Colley, 1989). Only recently have homologies within glycosyltransferase families been identified. Within the ST family comparison of the amino acid sequences in the catalytic domain of α -2,6-ST and α -2,3-ST revealed a

stretch of 45 amino acids with 65% identity (Grundmann *et al.*, 1990; Gillespie *et al.*, 1992). Among the human fucosyltransferases (FucT), α -1,3-FucT VI shows 85–89% sequence identity at the C-terminal region with the FucT III and FucT V but is very different from the myeloid type FucT IV (Weston *et al.*, 1992). Amino acid sequence homologies have also been identified among the transferases specific for the ABO blood system. Cloned cDNAs for these transferases show 99% homologies in the coding regions of A and B alleles with only seven nucleotide substitutions which result in four amino acid changes (Yamamoto *et al.*, 1990). Regardless of sequence similarities within or across families these enzymes exhibit a common topology which consists of a short N-terminal cytoplasmic tail, a signal anchor domain followed by a stem region and a large catalytic domain exposed to the Golgi lumen. Proteolytic cleavage of the membrane-bound enzyme in the stem region yields a soluble form which consists mainly of the catalytic domain.

β -1,4-Galactosyltransferase (β -1,4-GT) transfers galactose from UDP-galactose to the non-reducing terminal N-acetylglucosamine (GlcNAc) residues of glycoproteins and glycolipids, generating a β -1,4 linkage (Hill *et al.*, 1968). In mammalian cells β -1,4-GT is primarily localized in the trans-cisternae of the Golgi in its membrane-bound form. However, it has also been localized at the surface of many cells where it mediates a variety of cell–cell and cell–matrix interactions (Shur and Roth, 1975; Shur, 1984; Bayna *et al.*, 1988) and has been implicated as a mediator of growth control (Roth and White, 1972; Masibay *et al.*, 1991). In addition, soluble forms of this enzyme exist in milk, colostrum and serum which are derived from the membrane-bound form by proteolytic cleavage (Strous, 1986). The two secretory forms of bovine β -1,4-GT lack the first 78 or 96 amino acid residues of the full length protein (D'Agostaro *et al.*, 1989). The missing residues in the soluble forms of β -1,4-GT correspond to the N-terminal cytoplasmic tail, the transmembrane region and a portion of the stem region.

The functional domains of β -1,4-GT are being studied in various laboratories by expressing cDNA clones in both mammalian and bacterial cells (Masibay and Qasba, 1989; Aoki *et al.*, 1990; Nakazawa *et al.*, 1990). We have shown that the transmembrane domain is required for the stability and Golgi retention of β -1,4-GT in mammalian cells (Masibay *et al.*, 1992, 1993). The N-terminal sequence (amino acids 1–78 or 1–96) (D'Agostaro *et al.*, 1989), absent in the soluble forms of β -1,4-GT, is not necessary for the catalytic function which appears to be composed of two domains (Yadav and Brew, 1990, 1991). In this report we describe the expression of N-terminal deleted forms of bovine β -1,4-GT in *Escherichia coli* as fusion proteins, joined at the C-terminus of glutathione-S-transferase (GST), and describe a method that generates an enzymatically active β -1,4-GT. We show that deletion of residues 1–129 does not affect β -1,4-GT and lactose synthetase (LS) activities and that integrity of the disulfide bond, involving Cys134, is essential for proper folding and regeneration of the enzyme activity.

Materials and methods

Materials

Restriction and other modifying enzymes were purchased from New England Biolabs. [32 P]dCTP (>3000 Ci/mmol) was obtained from Amersham. A polymerase chain reaction (PCR) kit was purchased from Perkin Elmer Cetus Corp. Oligonucleotides for PCR were synthesized by Genosys Biotechnologies Inc. Glutathione–Sephadex 4B, isopropyl- β -D-thiogalactopyranoside (IPTG) and pGEX-2T vector were purchased from Pharmacia. Thrombin, glutathione, uridine 5'-diphosphohexanolamine (UDP–agarose) and 6-aminoethyl 2-acetamido-2-deoxy- β -D-thioglucopyranoside (GlcNAc–agarose) were obtained from Sigma. *Escherichia coli* strain JM109 was purchased from Stratagene. AG 1-X8 resin, chloride form, 200–400 mesh was obtained from Bio-Rad. Dithiothreitol (DTT) and phenylmethylsulfonyl fluoride (PMSF) were purchased from Boehringer Mannheim Biochem.

Methods

Cloning of β -1,4-GT cDNA fragments into pGEX-2T expression vector. General DNA recombinant techniques were carried out as described in *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1987). The β -1,4-GT sequence in pLbGT-1 (Narimatsu *et al.*, 1986) was either digested with restriction enzymes or PCR amplified and then subcloned into the polylinker site of pGEX-2T expression vector. For cloning purposes the 5' end oligonucleotide primers used in PCR contained a *Bam*HI site and two additional nucleotides (CG) at their 5' ends (Figure 1). The 3' end primer sequence corresponded to the unique *Eco*RI site in the non-coding region of β -1,4-GT in pLbGT-1. PCR amplification was carried out for 25 cycles, each cycle consisting of 1 min at 96°C, 2 min at 50°C and 5 min at 72°C with segment extension of 20 s/cycle. An aliquot of the PCR amplified DNA fragments was analyzed by electrophoresis on a 1% agarose gel in Tris–acetate–EDTA (TAE) buffer and visualized after ethidium bromide staining. PCR products were purified, digested with *Bam*HI/*Eco*RI and subcloned into the *Bam*HI/*Eco*RI site of the phosphatase-treated pGEX-2T and transfected into competent bacterial cells.

Cell culture and expression of β -1,4-GT. *Escherichia coli* cells harboring the recombinant plasmids were incubated overnight at 37°C in Luria–Bertani (LB) broth containing ampicillin (100 μ g/ml). An aliquot of the overnight culture was diluted 1:10 in fresh medium containing the antibiotic and incubated for 1 h at 37°C to early log phase ($A_{600} = 0.4$). Isopropyl- β -D-thiogalactopyranoside was added to a final concentration of 0.3 mM to induce the synthesis of the fusion proteins. Bacterial cultures were incubated for an additional 5 h. Cells were harvested by centrifugation at 4000 g for 10 min and the pellets stored at –70°C.

Isolation and purification of inclusion bodies. Inclusion bodies were isolated from bacterial pellets following the methods described by Smith and Johnson (1988) and Lin and Cheng (1991) with certain modifications. Frozen cell pellets from 250 ml cultures were thawed at room temperature and resuspended in 12.5 ml of 20 mM Tris–HCl, pH 7.5, 20% sucrose, 1 mM EDTA. After incubation on ice for 10 min bacteria were pelleted by centrifugation at 4000 g and resuspended in 12.5 ml of ice cold water for 10 min. Hypotonic treatment was followed by precipitation of the spheroplasts at 8000 g. Pellets were resuspended in 2.5 ml of suspension buffer (PBS, 5 mM EDTA,

1 μ g/ml leupeptin, 20 μ g/ml aprotinin and 0.5 mM PMSF). The cells were lysed by a sonicator three times with a 30 s pulse and incubated at room temperature for 10 min. The resulting suspensions were diluted with 10 ml of suspension buffer and the inclusion bodies fractionated by centrifugation at 13 000 g for 30 min. The pellet fraction (inclusion bodies) was carefully resuspended in 10 ml of washing buffer (PBS, 25% sucrose, 5 mM EDTA, 1% Triton X-100, 1 μ g/ml leupeptin and 0.5 mM PMSF), incubated on ice for 10 min and centrifuged for 10 min at 25 000 g. This washing step was repeated three more times and the washed pellet further processed.

Solubilization of inclusion bodies and renaturation of fusion proteins. The inclusion bodies were resuspended in 2.5 ml denaturation buffer (50 mM Tris–HCl, pH 8.0, 5 M guanidine HCl, 5 mM EDTA). Protein aggregates were disrupted by sonication with a 5 s pulse, incubated on ice for 1 h and centrifuged at 12 000 g for 30 min. The optimum conditions for renaturation of the fusion proteins were determined and are as follows: 5 M guanidine HCl solubilized protein (2–5 mg/ml) was added drop-wise to a 10-fold volume of cold buffer (50 mM Tris–HCl, pH 8.0, 1% Triton X-100, 8:1 mM GSH:GSSG, 1 μ g/ml leupeptin, 0.5 mM PMSF) that was constantly vortexed during the addition of the protein. The sample was gently agitated overnight at 4°C and the renatured protein dialyzed against 50 mM Tris–HCl pH 8.0 that contained 1% Triton X-100 and protease inhibitors.

Affinity purification of recombinant fusion proteins. Renatured fusion proteins were affinity purified using glutathione–Sephadex 4B columns prepared according to the manufacturers directions. Renatured proteins derived from 250 ml cultures were diluted 3-fold in dialysis buffer and applied to the columns (1 ml bed volume). The bound proteins were eluted with 5 mM reduced glutathione in 10 fractions of 0.5 ml each and subsequently cleaved with thrombin. Alternatively, bound material was cleaved directly on the column in 1 ml of buffer containing 0.4 U of thrombin and then eluted to obtain proteins devoid of the GST domain. The cleaved proteins were tested for their capacity to bind UDP– and GlcNAc–agarose columns as previously described (Barker *et al.*, 1972). The purity of the recombinant proteins was judged on SDS gels.

Protein estimation and SDS–polyacrylamide gel electrophoresis. The protein concentration of each sample was measured using the Bio-Rad Protein Assay-dye reagent concentrate (Bio-Rad Laboratories) with bovine serum albumin (BSA) as standard. Proteins were analyzed by SDS–PAGE under reduced conditions using pre-cast 10% gels from Novex. The proteins in the gels were visualized with Coomassie brilliant blue R-250.

β -1,4-GT and LS assays. β -1,4-GT and LS assays were performed at 30°C for 30 min in 100 μ l incubation mixtures containing 2.5 μ mol of Tris–HCl, pH 8.0, 5 nmol of UDP-galactose, 0.5 μ Ci UDP- 32 H-galactose (~19 Ci/mmol), 400 nmol of MnCl₂ and 0.5 μ l of Triton X-100 in the presence or absence of 2 μ mol GlcNAc (β -1,4-GT assay) or 2 μ mol glucose (LS assay) as the acceptor molecules. Under these assay conditions there are typically $1.75\text{--}2.0 \times 10^5$ c.p.m./nmol of UDP-galactose. The 32 H-labeled products were separated on an anion-exchange column (AG 1-X8) as previously described (Qasba and Chakrabarty, 1978). Incorporation was linear for 25–30 min. For kinetic analysis incubations were carried out

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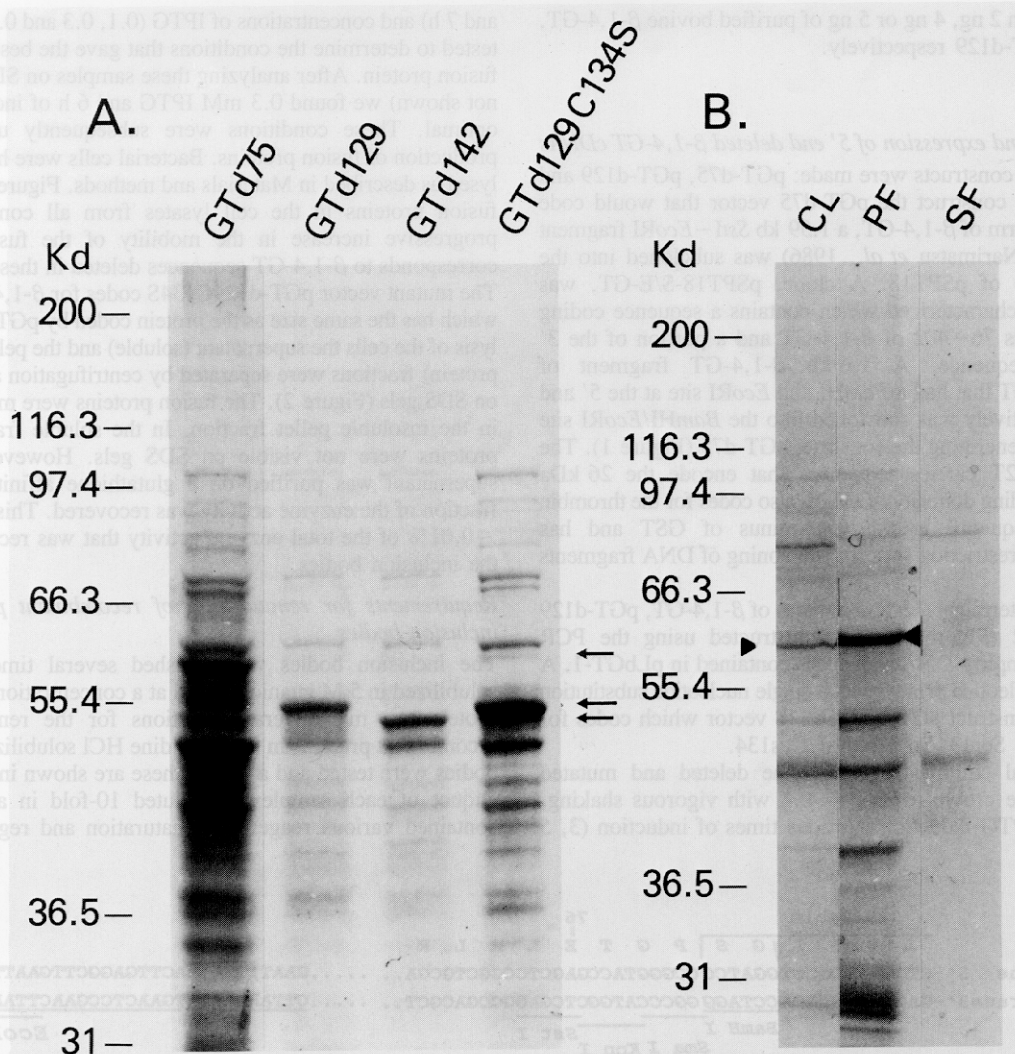


Fig. 2. SDS-PAGE analysis of bacterial lysates from IPTG-induced clones expressing bovine β -1,4-galactosyltransferase. **(A)** Bacterial cultures from pGT-d75, pGT-d129, pGT-d142 and pGT-d129C134S were induced with IPTG and lysed in SDS sample buffer. The lysed samples were electrophoresed on a 10% gel and stained with Coomassie blue R-250. Protein standard markers (kDa). Arrows point to induced proteins. **(B)** Bacterial cell pellets from IPTG-induced pGT-d75 were resuspended in lysis buffer and centrifuged at 13 000 g. Proteins from total cell lysates (CL), from insoluble pellet (PF) and from supernatant fractions (SF) were analyzed on 10% gels. Arrows point to induced proteins.

β -1,4-GT activity was absolutely dependent on a mixture of reduced and oxidized glutathione. The protein precipitated out during overnight renaturation and showed no enzyme activity when the 'oxido-shuffling' (Rudolph, 1990) reagent was absent even though glycerol (or Triton X-100) was present. The activity of the protein was dependent on oxidized and reduced glutathione and increased as the glycerol concentration was reduced from 20 to 5%. The maximal activity was obtained when the renaturation buffer contained at least 1% Triton X-100, reduced and oxidized glutathione in a ratio of 8:1 mM and the pH of the solution was 8.0. High glycerol concentration does not inhibit the enzyme activity but appears to interfere with the refolding process. Table I shows that even in the absence of glycerol neither MnCl_2 nor UDP-galactose are required during the renaturation process to regenerate the enzyme activity. Treatment of the solubilized inclusion bodies with DTT prior to renaturation did not increase β -1,4-GT activity. The renatured samples showed a 2-fold increase in enzyme activity after dialysis. SDS gel

analysis of the dialyzed samples shows that the major protein band corresponds to the fusion protein. At this stage of purification the fusion protein could be cleaved with thrombin, releasing the GST domain (Figure 3).

Glutathione affinity purification of β -1,4-GT fusion proteins

Prior to binding to the glutathione columns equal amounts of fusion proteins were observed on SDS gels in the samples from the various constructs (Figure 2). The renatured fusion proteins from pGT-d75 and pGT-d129 bind to glutathione-Sepharose and can be purified on these columns (Figure 3A). However, the renatured fusion protein from pGT-d142, that lacks residues 1–142 of β -1,4-GT, and the fusion protein from pGT-d129C134S show a lower binding affinity to the glutathione-Sepharose column. The GST domain can be directly removed from the β -1,4-GT fusion protein bound to glutathione-Sepharose by treating the matrix with thrombin and then eluting the recombinant protein from the column

Table I. Composition of buffers during renaturation of β -1,4-galactosyltransferase fusion protein

GSH:GSSG (mM)	MnCl ₂ (100 μ M)	UDP-Gal (50 μ M)	DTT (1 mM)	Glycerol (%)	Triton X-100	Tris pH (50 mM)	Activity (%)
0:0	+	—	+	20	—	8	1
8:1	—	—	—	20	—	8	55
8:1	—	—	—	5	—	8	82
8:1	—	—	—	—	2	8	100

An aliquot of guanidine HCl solubilized protein was added drop-wise to a 10-fold volume of buffer with constant vortexing. The composition of each buffer is given above. The sample was assayed for β -1,4-GT activity after overnight renaturation at 4°C.

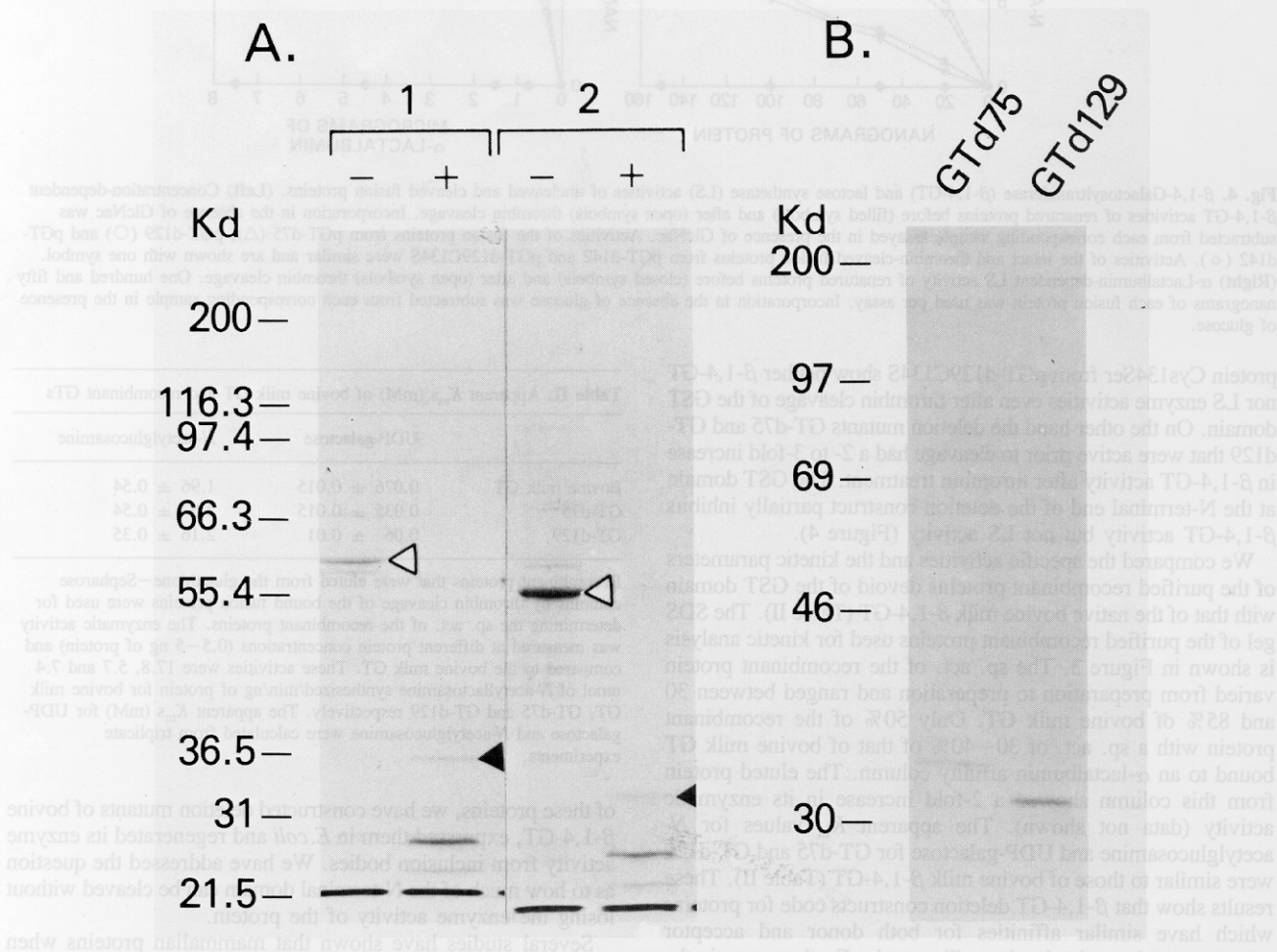


Fig. 3. Analysis of renatured and glutathione affinity purified fusion proteins before and after thrombin cleavage. (A) Insoluble bacterial fractions containing inclusion bodies were solubilized in denaturing buffer and the proteins refolded under renaturing conditions. The samples were dialyzed, cleaved with 0.4 U of thrombin and analyzed on 10% SDS gels. Fusion proteins from pGT-d75 (lane 1) and pGT-d129 (lane 2), uncleaved (—) and cleaved (+). Open triangles (Δ) and closed triangles (\blacktriangle) point to uncleaved and cleaved protein bands respectively. (B) The renatured and dialyzed fusion proteins were bound to glutathione–Sephacrose 4B. Overnight treatment of the column-bound samples with thrombin resulted in cleavage of the recombinant GT fused at the C-end of GST. An aliquot of the eluted proteins devoid of the GST domain was subjected to electrophoresis under reducing conditions. Protein standards, recombinant GT protein from pGT-d75 and pGT-d129.

(Figure 3B). The final yield of the fusion protein from deletion constructs pGT-d75 and pGT-d129 was ~ 2 mg/l of bacterial culture. The percentage yield of the renatured enzyme was determined by SDS–PAGE analysis of the protein before and after binding to glutathione–Sephacrose columns. Roughly 80% of the native enzyme from the inclusion bodies bound to the matrix. Both uncleaved and thrombin-cleaved fusion proteins were further analyzed for β -1,4-GT and LS activities (Figure 4).

Enzymatic activity of deletion constructs and Cys134 mutants

Concentration-dependent galactosyltransferase and α -lactalbumin-dependent lactose synthetase activities of the deleted and mutated fusion proteins before and after cleavage with thrombin are shown in Figure 4. Fusion proteins derived from pGT-d75 and pGT-d129 were active in β -1,4-GT and LS assays. The fusion protein from pGT-d142, which lacks residues 1–142, and the mutant

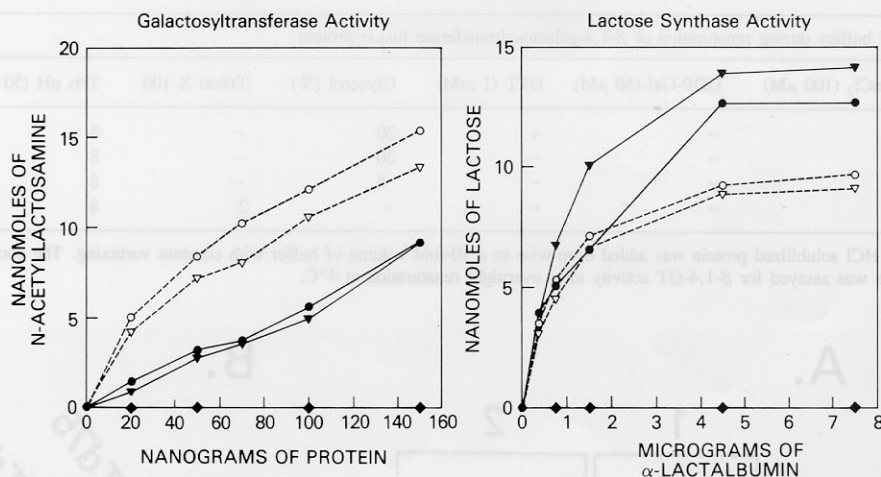


Fig. 4. β -1,4-Galactosyltransferase (β -1,4-GT) and lactose synthetase (LS) activities of uncleaved and cleaved fusion proteins. **(Left)** Concentration-dependent β -1,4-GT activities of renatured proteins before (filled symbols) and after (open symbols) thrombin cleavage. Incorporation in the absence of GlcNAc was subtracted from each corresponding sample assayed in the presence of GlcNAc. Activities of the fusion proteins from pGT-d75 (Δ), pGT-d129 (\circ) and pGT-d142 (\diamond). Activities of the intact and thrombin-cleaved fusion proteins from pGT-d142 and pGT-d129C134S were similar and are shown with one symbol. **(Right)** α -Lactalbumin-dependent LS activity of renatured proteins before (closed symbols) and after (open symbols) thrombin cleavage. One hundred and fifty nanograms of each fusion protein was used per assay. Incorporation in the absence of glucose was subtracted from each corresponding sample in the presence of glucose.

protein Cys134Ser from pGT-d129C134S show neither β -1,4-GT nor LS enzyme activities even after thrombin cleavage of the GST domain. On the other hand the deletion mutants GT-d75 and GT-d129 that were active prior to cleavage had a 2- to 3-fold increase in β -1,4-GT activity after thrombin treatment. The GST domain at the N-terminal end of the deletion construct partially inhibits β -1,4-GT activity but not LS activity (Figure 4).

We compared the specific activities and the kinetic parameters of the purified recombinant proteins devoid of the GST domain with that of the native bovine milk β -1,4-GT (Table II). The SDS gel of the purified recombinant proteins used for kinetic analysis is shown in Figure 3. The sp. act. of the recombinant protein varied from preparation to preparation and ranged between 30 and 85% of bovine milk GT. Only 50% of the recombinant protein with a sp. act. of 30–40% of that of bovine milk GT bound to an α -lactalbumin affinity column. The eluted protein from this column showed a 2-fold increase in its enzymatic activity (data not shown). The apparent K_m values for N -acetylglucosamine and UDP-galactose for GT-d75 and GT-d129 were similar to those of bovine milk β -1,4-GT (Table II). These results show that β -1,4-GT deletion constructs code for proteins which have similar affinities for both donor and acceptor substrates as the native bovine milk protein. Furthermore, in the presence of Mn^{2+} both GT-d75 and GT-d129 proteins bind to UDP- and GlcNAc-agarose columns and can be eluted with 5 mM GlcNAc (data not shown). These results show that residues 1–129 are not involved in the binding of either UDP-galactose or N -acetylglucosamine.

Discussion

Several studies on the molecular cloning of glycosyltransferases have revealed that these enzymes share a common topology but little sequence similarity. The catalytic domain of these enzymes is present at the C-terminal region of the protein. The secreted forms of these proteins, which are enzymatically active, lack a substantial portion of the N-terminal region present in the precursor protein. To study the structure–function relationship

Table II. Apparent K_m s (mM) of bovine milk GT and recombinant GTs

	UDP-galactose	N -acetylglucosamine
Bovine milk GT	0.076 ± 0.015	1.96 ± 0.54
GT-d75	0.035 ± 0.015	2.57 ± 0.54
GT-d129	0.06 ± 0.01	2.16 ± 0.35

Recombinant proteins that were eluted from the glutathione–Sephacrose columns by thrombin cleavage of the bound fusion proteins were used for determining the sp. act. of the recombinant proteins. The enzymatic activity was measured at different protein concentrations (0.5–5 ng of protein) and compared to the bovine milk GT. These activities were 17.8, 5.7 and 7.4 nmol of N -acetyllactosamine synthesized/min/ng of protein for bovine milk GT, GT-d75 and GT-d129 respectively. The apparent K_m s (mM) for UDP-galactose and N -acetylglucosamine were calculated from triplicate experiments.

of these proteins, we have constructed deletion mutants of bovine β -1,4-GT, expressed them in *E. coli* and regenerated its enzyme activity from inclusion bodies. We have addressed the question as to how much of the N-terminal domain can be cleaved without losing the enzyme activity of the protein.

Several studies have shown that mammalian proteins when expressed in *E. coli* aggregate and form inclusion bodies (Wang *et al.*, 1989; Hartman *et al.*, 1992; Kohnert *et al.*, 1992; Taylor *et al.*, 1992). Our results show that β -1,4-GT expressed as GST fusion protein in *E. coli* also forms inclusion bodies. The inclusion bodies can be solubilized in 5 M guanidine HCl and the enzyme activity of the protein can be regenerated only in the presence of an 'oxido-shuffling' reagent and at least 1% Triton X-100. During this renaturation step both the GST domain and the recombinant β -1,4-GT fold into a conformation which allows it to bind to the glutathione affinity column. The recombinant proteins GT-d75 and GT-d129, devoid of GST domain, obtained after thrombin treatment of the column-bound fusion proteins are 30–85% as active as the β -1,4-GT bovine milk GT, with similar Michaelis constants for N -acetylglucosamine and UDP-galactose. These β -1,4-GT proteins bind to UDP- and GlcNAc-agarose columns which shows that the N-terminal residues 1–129 of

β -1,4-GT are not necessary either for β -1,4-GT or for LS activities and furthermore suggests that the carbohydrate moiety attached to the native β -1,4-GT does not influence its activity. The removal of residues 1–142, which includes Cys at position 134, abrogates both enzyme activities. Site-directed mutagenesis of Cys134 to Ser in pGT-d129 or to Ala (data not shown) also abolishes β -1,4-GT and LS activities. Recently Yadav and Brew (1991) showed that two of the five cysteine residues in the secreted form of β -1,4-GT are involved in the formation of a disulfide bond (Cys134 and 247) and have suggested that GT protein consists of two domains: one domain (residues 79–250) interacts with α -lactalbumin and the other domain is involved in the binding of UDP-galactose and *N*-acetylglucosamine (residues 275–402). The requirement of 'oxido-shuffling' reagent to regenerate β -1,4-GT or LS activity, together with the observation that mutating Cys134 leads to an inactive enzyme, confirms the importance of the disulfide bond between Cys134 and Cys247 for proper folding and enzymatic function of the protein.

The secreted form of human β -1,4-GT has previously been expressed in the periplasmic space of *E. coli* (Aoki *et al.*, 1990). These studies showed that the activity was detectable in the culture medium and in the periplasmic fraction but the protein could not be visualized in gels; we also detected enzymatic activity in the soluble fraction and could not visualize the protein in gels. The enzyme activity that can be recovered from the supernatant fraction represents only 0.01% of the total activity that can be recovered from the inclusion bodies. The active protein in the supernatant represents a fraction of the protein that undergoes disulfide bond formation either in *E. coli* or during purification and does not require any 'oxido-shuffling' reagent. The soluble fractions of pGT-d142 and pGT-d129C134S did not show any enzyme activities, which is consistent with the hypothesis that the integrity of the disulfide bond between Cys134 and Cys247 is essential to hold the protein in a conformation required for generating active enzyme. In addition our results show that nearly 99% of the fusion protein produced in *E. coli* using pGEX-2T vector is present in inclusion bodies and requires an 'oxido-shuffling' reagent for the regeneration of its activity. The final yield of the renatured and active β -1,4-GT recovered from the inclusion bodies devoid of GST domain is nearly 1 mg/l of bacterial culture.

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